Current Topics

Mighty Microbes: Bacterial Bonding Is Plenty Powerful

Bacteria may be tiny from a human’s vantage point, but size is no indication of strength, according to David Popham of Virginia Polytechnic Institute and State University, Blacksburg, who spoke during the symposium “Forces Generated by Bacteria” at the 106th ASM General Meeting in Orlando last May. On a per-mass basis, some bacteria adhere with a force that is 10- to 100-fold greater than a leaping human, he says.

Some bacterial pathogens adhere tenaciously to host cell surfaces, while others hold fast to medical devices to establish a foothold in hosts. For example, Staphylococcus aureus cells form biofilms that strongly adhere to medical devices, helping to account for many of the 1 million nosocomial infections per year in the United States that are associated with the use of such indwelling devices, according to symposium participant Steven Lower of the Ohio State University in Columbus.

Using atomic force microscopy equipped with a fibronectin-coated probe, Lower and his collaborators determined that ordinary S. aureus cells only weakly adhere to coated probe surfaces—with forces in the nanoNewton range. However, many implant infection-associated S. aureus strains, some of which overexpress an outer-membrane fibronectin-binding protein, adhere to such probes more tightly. In one series of tests with clinical isolates, 60% of troublemaker strains measurably adhere to the probe, compared with 29% of controls.

The pathogenic cells exert a wax-waning force pattern that, when graphed, is shaped like sawblade teeth. S. aureus lacking the fibronectin-binding protein, as well as bacteria obtained from asymptomatic cardiac implant growths, display a different pattern, with an initial resistance that quickly dissipates as the probe is retracted.

The significance of the “sawtooth pattern” is still under study. Lower and colleagues think that different domains within the fibronectin-binding protein may be involved in binding to fibronectin. As some domains lose their binding strength, other portions of the protein may still bind strongly to surfaces. “Domains are unraveling within fibronectin-binding proteins such that the bond is more robust,” he explains. “It looks like [fibronectin binding] is not the only thing that is important when a bond forms between staph and a fibronectin-coated surface.”

Meanwhile, type IV pili along the surface of Neisseria gonorrhoeae cells are essential for this pathogen’s capacity to adhere to and infect host cells. However, instead of acting singly, the pili form bundles that exert larger retraction forces than can solo-acting pili, according to Michael Sheetz of Columbia University in New York, N.Y.

Sheetz and his collaborators use laser tweezers—an infrared laser beam that can trap and manipulate particles (Microbe, July 2006, p. 330)—to measure adherent forces exerted by N. gonorrhoeae cells that attach to beads via flexible pillars. The laser tweezers also can measure forces exerted by a bacterium perched on one pillar attached via its pili to surrounding pillars, which twitch when the pili retract. Scanning and transmission electron microscopy (TEM) confirm that the pili anchor not by lone threads, but by ropelike bundles.

“Having bundles is advantageous since the force of retraction is enhanced,” Sheetz says.

TEM provides tantalizing hints that the pilus bundles associate with an electron-dense cytoplasmic structure. If so, the bacterial force of retraction could be a coordinated response to a surface, and blocking that response might interfere with pathogenesis, Sheetz speculates.

Brian Hoyle
Brian Hoyle is President of Square Rainbow Limited, a science writing and editing company located in Bedford, Nova Scotia, Canada.

Glassy-Winged Sharpshooters Depend on Two Endosymbionts

The fruit of the vine does not suffice for the voracious glassy-winged sharpshooter, an insect pest and vector of diseases that feeds on grapevines, citrus, and other plant species. Remarkably, this insect also very much depends on not one, but two bacterial endosymbionts—one supplying amino acids and the other, vitamins—to sustain itself, according to Jonathan Eisen of the University of California, Davis, and his collaborators, who report their findings in the June PLoS Biology.

“Up to this point, scientists have mostly focused on what you might call the ‘it-takes-two-to-tango’ model of symbiosis, where the eukaryote has its
requirements provided by a single bacterial symbiont,” comments Abigail Salyers of the University of Illinois, Champaign-Urbana. “In this case, it takes three to tango, with one bacterial endosymbiont producing amino acids and the other producing vitamins.” The symbiosis appears even more complicated because the two bacterial endosymbionts not only supply their insect hosts with nutrients, but may also “be producing them for each other,” she adds.

Glassy-winged sharpshooters are notorious in several U.S. wine-growing regions where they are dreaded for spreading Pierce’s disease among vineyards. That disease is attributed to another bacterium that these insects carry, *Xylella fastidiosa* (ASM News, June 2000, p. 328). Regardless of whether they cause disease, the sharpshooters feed on liquid flowing through the xylem of grape vines or other plants, a system that “is very nutrient poor and is missing many things that animals need from their diets,” Eisen says. Based on knowledge of aphids, which also feed on nutrient-poor plant tissues and depend on the endosymbiont *Baumannia cicadellinicola*, he and his collaborators suspected—correctly—that another *Baumannia* was supporting the sharpshooters.

Eisen, then at the Institute for Genomic Research in Rockville, Md., Nancy Moran of the University of Arizona, Tucson, and their collaborators isolated and sequenced DNA fragments from the sharpshooter tissues inhabited by *Baumannia*. Based on these data, they determined the base-pair sequence of the sharpshooter-associated *Baumannia* genome. Subsequent analysis indicated that, unlike the aphid symbiont, this *Baumannia* appears capable of producing vitamins but not the amino acids that are essential for sustaining the sharpshooters.

So where do the sharpshooters obtain those vital amino acids? From *Sulcia muelleri*, a second type of bacterial endosymbiont that Moran earlier found inhabiting some of the same insect tissues as *Baumannia*. Indeed, Moran and Eisen’s more recent genomic sequencing efforts produced data that did not correspond to *Baumannia*, and those discrepancies led the investigators to use a metagenomic method to sort sequences by taxonomy.

“All of the genes that could encode essential amino acid synthesis pathways and no vitamin and cofactor pathways [were there]. In essence, this was a mirror image of *Baumannia*.

“Endosymbionts are particularly interesting both because of the parallels with organelles but also because of the parallels with intracellular pathogens,” Eisen continues. “Why is one organism beneficial and another detrimental? Do pathogens attenuate over time and become mutualists? A general understanding of how such symbionts avoid being rejected by the immune system of hosts will help us better understand how some pathogens can do the same thing.”

Eisen views these efforts as a “triumph” for metagenomics, which involves studying mixed genomes obtained from environmental samples. At least four distinct organisms were in the samples being analyzed, and “even in this simple system the bioinformatics is very, very hard,” he says. “That’s why I think it can be a model system that we can use to learn things that can be applied to more complicated systems.”

David Holzman
David Holzman is the Microbe Journal Highlights Editor.

**Gut Microbiota Evince Surprising Suppleness, Influence**

The microbiota of gastrointestinal (GI) tracts—the guts—of various animal species react to surprising host cues and influence the health and metabolism of their hosts to an unexpected extent, including by “regulating the host energy balance,” according to Jeffrey Gordon of Washington University School of Medicine in St. Louis, Mo., and his collaborators. Gordon described experiments with zebrafish and mice during the colloquium “Environmental Metagenomics: Promise and Limitations” at the 106th ASM...
General Meeting in Orlando, Fla., last May. His collaborator Buck Samuel described how particular mixes of gut microbes in mice affect host energy metabolism and weight gain during the poster session “Ecology, Physiology, and Molecular Biology of Archaea.”

The Washington University researchers often use germ-free animals, and then introduce particular species of microorganisms to learn more about how those microbes behave in simpler mixes than occur naturally in GI tracts. For example, Samuel co-colonized the guts of mice with a mere two species of microorganisms and soon found that a particular archaeon-bacterium pair form a “mutualism,” that is, grow into a balanced population with an interrelationship that appears synergistic.

The balance between those two organisms, Methanobrevibacter smithii and Bacteroides thetaiotamicron, is by no means equal in terms of biomass and numbers, Samuel says. Although the methanogen remains a “minor component” of the gut flora of these mice, it is a “keystone species with a disproportionate impact.” The balance that these two kinds of microbes strike proves critical for the way that they deal with complex carbohydrates that are part of their mouse host’s diet. How the two microbes—or other pairs (and, presumably more complex microbial combinations)—metabolize dietary carbohydrates dramatically affects the mice, enabling them to extract more energy and to gain more weight from particular components in the diet, he points out. “M. smithii has dramatic effects on glycan fermentation, and enhances the degradation of fructans, modulating fermentation... toward acetate and formate.”

In simpler terms, the breakdown of dietary sugars becomes more thorough, enabling the mice carrying that particular pair of microbes to get fatter, faster than do mice carrying other microbial pairs, according to Samuel. The twosome extracts “otherwise indigestible waste products from fibers and complex polysaccharides, making gas and short-chain fatty acids... up to 10% of the caloric intake,” he says. And, although the mice are both fatter and gassier than usual, they are “generally healthy.”

The mix of microbiota, or their absence, influences feed efficiency and energy recovery by the host, according to Gordon, who points to other experiments in this series. For example, he notes, the guts of obese mice “have more firmicutes than Bacteroides species” than do the guts of their leaner littermates. Rather than being an adaptation to a change in diet, this shift in balance within the mouse gut microbiota is “associated with an increase in capacity for harvesting energy,” he says.

Gut microbiota hold sway in other ways when introduced into germ-free animals, including zebrafish and mice, according to Gordon. In general, the gut microbiota amounts to highly complex mixtures of hundreds of strains and species, albeit dominated by bacteroides and firmicutes while having minority populations, including archaeal species.

When the usual mix of gut microbiota from zebras are “transplanted” into mice, the firmicutes in the GI tracts of those recipient mice “expand and become dominant,” Gordon says. “When the mouse microbiota is transplanted into zebrafish, the mouse-derived proteobacteria expand dramatically.” Somehow, he points out, “the host gut environment selects what it gets... in predictable ways.”

One reason for this apparent host environment-directed selectivity is to “modulate” important metabolic activities, including those of the immune system, that are important to the health of that individual host and, more than likely, the species to which it belongs.

In a related development, Gordon and collaborators, including Karen Nelson and Steven Gill at the Institute for Genomic Research in Rockville, Md., recently reported that the human gut microbiota contains as many as 100 trillion microbes from more than...
1,000 species, most belonging to the Firmicutes and Actinobacteria, but also containing the methanogenic archaeon M. smithii. Resident microbes in the human colon actively synthesize vitamins and break down plant sugars such as xylan and celllobiose that humans do not otherwise digest. Additional details appear in the June 2 issue of Science.

Jeffrey L. Fox is the Microbe Current Topics and Features Editor.

**σ^E** Added to ppGpp- Coordinated Stress Responses in *E. coli*

Add σ^E to the list of alternative sigma factors, that is, subunits of RNA polymerase, that help Escherichia coli cells mediate stress responses. In *E. coli*, the small molecule ppGpp triggers the release of σ^E, according to Alessandra Costanzo and Sarah E. Ades of the Pennsylvania State University, University Park, whose findings appear in the July issue of *Journal of Bacteriology* (188:4627–4634).

Sigma factors such as σ^E redirect cellular transcriptional machinery to specific promoters, thereby shifting protein synthesis to better respond to specific stressors, such as starvation and heat shock. In some cases, these specialized alternative sigma factors are part of the cell response to specific stresses. In the case of σ^E, the response is to damage of specific components in the cell envelope, namely outer membrane porins, which are transmembrane proteins that control the entry of sugars, ions, and amino acids. In Salmonella, by contrast, σ^E is activated during stationary phase and when cells are starved for carbon-containing nutrients.

Thus, similar types of bacterial species respond in distinct ways to environmental stresses, but at least in the case of *E. coli* that response is efficient, according to Costanzo and Ades. When porins are damaged, they unfold and quickly activate a proteolytic cascade that degrades an inhibitor of σ^E, called RseA. This new activation pathway also involves ppGpp, a small molecule whose levels become elevated as a sign of nutritional stress. ppGpp also activates at least two other alternative sigma factors when cells enter stationary phase: σ^N, a general stress factor, and σ^S, which is associated with genes encoding nitrogen-sensing proteins.

“Our work adds σ^E to this list, which means that every alternative sigma factor tested thus far (there are six in *E. coli*) can be activated by ppGpp,” Ades says. This is important because, outside the laboratory, bacteria encounter stresses in bunches. In *E. coli*, ppGpp enables cells to address many of those stresses all at once. “An analogy would be that during an emergency, it is more efficient to sound an alarm (ppGpp) and prepare all civil defense [branches] at once rather than waiting for flooding to call the people to repair the levees, waiting for a food shortage to call for food donations, and waiting for looting to call the police,” she says. “We believe that this system exists to make the cell envelope more stress resistant during times of nutrient scarcity rather than waiting until the cell envelope is damaged to try to repair it.”

“The research is important in a fundamental way, in elucidating the mechanisms by which environmental signals are transduced by cells and used to trigger adaptive responses,” says Ferric C. Fang of the University of Washington, Seattle. It also has practical implications, he adds, by providing “an increased understanding of the ways in which pathogenic bacteria can resist immune defenses and the action of antibiotics. In bacteria other than *E. coli*, the σ^E regulon has been shown to be essential for virulence.” σ^E-deficient mutants are unusually susceptible to mediators of innate immunity, and to antimicrobial peptides and other antibiotics, he notes.

Additionally, elevated ppGpp is linked to antibiotic resistance that occurs when cells stop dividing, according to Ades. Apparently, ppGpp triggers cells to stop growing and may also upregulate stress responses, making them no longer susceptible to antibiotics such as penicillin.

David Holzman
Assessing Virulence of Prions in Yeast Models

Insights about prions come from a study published on 28 June in Nature by Jonathan Weissman of the University of California, San Francisco, and his collaborators. They find that the virulence of prions—at least, those from yeast—apparently is based more on their fragility than on their speed of replication. Thus, the slowest-growing conformation of a particular yeast prion seemed to have the strongest effect in producing protein aggregates inside yeast cells, he says. However, the slower growth of that conformation was more than compensated for by an increased brittleness that promotes fragmentation, and thus proliferation of the prions. “From a therapeutic point of view, our findings suggest that effective treatment strategies for prion diseases might aim at stabilizing prion aggregates,” he says.

MicroRNA May Account for Latency Effects in Herpes-Infected Cells

Although the herpes simplex-1 virus (HSV-1) is sensitive to drugs and immune system responses while actively infecting mammalian host cells, those vulnerabilities vanish when it becomes latent in nerve cells only to reappear once infectivity resumes. The key to some of these effects apparently resides in the viral gene, called latency-associated transcript (LAT), that is the only viral gene expressed during latency. Surprisingly, LAT encodes a regulatory microRNA (miRNA) instead of a protein, albeit one that was (vainly) sought, according to Nigel Fraser at the University of Pennsylvania (UP) School of Medicine in Philadelphia and his collaborators, whose findings appear in the May 31, 2006 issue of Nature. “When you’re banging your head against the wall, you try a different direction,” he says.

HSV-1 latency is a “passive phenomenon,” one that “results from the lack of activation of the acute infection,” Fraser says. The newly recognized miRNA “offers the first possibility for new therapeutics to treat latent herpes infections.” For example, although acyclovir disrupts viral DNA replication and “works wonderfully on growing viruses, there are no targets for latent viruses,” he adds.

In general, miRNA molecules control cellular processes by regulating the translation of messenger RNA molecules. Those miRNA molecules themselves are spliced from longer transcripts by specialized enzymes in the nucleus, called drosher, and in the cytoplasm, called dicer. In the case of LAT, the cell process at issue is programmed cell death, also called apoptosis, and the specific miRNA is generated from the exon 1 region of the LAT HSV-1 gene.

Several years ago, Steve Wechsler at the University of California, Irvine, and his collaborators determined that LAT somehow blocks apoptosis in nerve cells. Although they concluded that LAT did not encode a protein and speculated that RNA may block apoptosis, “we couldn’t figure out a way to look at the RNA directly,” he says. He calls Fraser’s findings “very exciting” because they “demonstrate that RNA blocks apoptosis without making a protein.”

After confirming that LAT can confer resistance to apoptosis in neuroblastoma cells in vitro, Fraser and his collaborators inserted a specific, short interfering RNA molecule that turns off the dicer enzyme in such cells. When the drug cisplatin was added to trigger apoptosis, only those neuroblastoma cells lacking the dicer enzyme died, whereas cells retaining dicer survived. In addition, a mutant virus missing the exon 1 fragment that generates LAT neither protects cells from cisplatin-induced death nor generates miRNA. Further evidence suggests that the HSV-1 LAT gene affects several signaling pathways involved in apoptosis of nerve cells, thus allowing HSV-1 to survive latently in infected cells.

The discovery of the LAT miRNA of course explains why no one could find a viral protein expressed by HSV-1 LAT during latent infections. Meanwhile, some of the other UP experiments could help to explain how this virus evades the host immune system. Nonetheless, “this may not be the whole answer,” Wechsler points out. Considering that the LAT transcript is four times larger on average than other HSV genes and is highly conserved, other regions in LAT might also be involved in latency, he speculates. Hence, he, Fraser, their respective collaborators, and others are continuing to look for other potentially important miRNAs in HSV-1, HSV-2, and other viruses capable of latency.

Carol Potera
Carol Potera is a science writer in Great Falls, Mont.

Tracking Effects of Bacterial Toxins in Zebrafish Embryos

Clostridium difficile bacteria produce two principal toxins that exert systemic effects on infected animal hosts. One way to learn what organs and tissues those toxins target is to follow their effects in zebrafish embryos, according to Jimmy Ballard from the University of Oklahoma Health Sciences Center in Oklahoma City. He spoke during the symposium “Emerging Models in Toxin-Host Interactions” at the 106th ASM General Meeting in Orlando, Fla., last May.

C. difficile causes infections among
more than 300,000 hospitalized patients per year in the United States, leading to complications that cost more than $1 billion to treat, according to Ballard. Some hypervirulent strains of this pathogen produce unusually high levels of toxins and cause more medical havoc than do ordinary strains. Toxin A is believed to be mainly responsible for the gastrointestinal (GI) effects associated with such infections.

Meanwhile, the effects of toxin B, which he calls “very potent,” appear to be both systemic and cytotoxic, and may lead to organ failure and shock, Ballard says.

However, precisely where this toxin acts and what organs it targets in humans are largely mysterious. Although investigators have analyzed the effects of this toxin on rodents, those efforts typically entail postmortem inspections. However, because the embryos of zebrafish are transparent, one can track the effects of toxin B of *C. difficile* or other such toxins “visually, as they happen,” Ballard says. Indeed, toxin B localizes to the pericardial region of the zebrafish embryo, quickly leading to “pronounced edema,” or swelling, as fluid collects around the heart. Also, when the toxin is added to such fish, “blood flow is substantially decreased . . . and the heart becomes very labored in its beating.”

Some of these same effects are seen when the toxin is applied to rat heart cells (cardiomyocytes) that are growing in tissue culture, helping to confirm that it targets similar organs in mammals, according to Ballard. Thus, after the toxin is added to such cells, they round up, become increasingly disorganized, and lose their formerly rhythmic beating. Moreover, the toxin appears to target the hearts of mice and, when administered to such animals, leads to inflammation, edema, and other damage to their hearts, he says. Similarly, when hams ters are infected with *C. difficile*, the hearts of those animals also are damaged. However, whether that damage is directly attributable to toxin B remains to be proved, he notes.

In principle, the zebrafish also provide a means for rapidly testing some of the effects of candidate drugs, Ballard points out. Other efforts indicate that toxin B, also known as TcdB, triggers apoptosis (programmed cell death) by working through a cell fac-

**Jeffrey L. Fox**

**Aeromonas: Jack-of-All-Trades Pathogen with a “Rodney Dangerfield” Complex**

“We think of *Aeromonas* as the jack-of-all-trades [among pathogens]—more so than *Acinetobacter*,” says Amy Horneman of the University of Maryland (UM) School of Medicine in Baltimore. Based on the recent analysis of the *Aeromonas* genome, its gene profiles resemble those of several other more notorious pathogens, including *Vibrio* and *Pseudomonas*, she points out. Horneman was one of several experts who argued that this pathogenic microbe is simply not given the credit it deserves during the symposium “Aeromonas and *Plesiomonas*: a Pair of Underappreciated Emerging Pathogens with a ‘Rodney Dangerfield’ Complex” at the 106th ASM General Meeting in Orlando, Fla., last May.

Sam Joseph of the University of
NIAID Program To Model Immune Responses to Infectious Diseases

Officials at the National Institute of Allergy and Infectious Diseases (NIAID) in July announced the new Program in Systems Immunology and Infectious Disease Modeling (PSIIM) that will use computational systems biology to develop a deeper understanding of how pathogens cause disease and how the immune system responds to them. The cornerstone of the PSIIM research project is a software package called Simmune that was developed by Martin Meier-Schellersheim of NIAID and his collaborators. Simmune uses quantitative data and provides a graphical interface to depict interactions between individual molecules in a large network or the behaviors of cells in response to external signals. The NIAID scientists recently used Simmune to model cell-level chemosensing, and how levels of membrane phospholipids at the “front,” or sensing, end of 9 cell increase during this process. They report that experimental data match closely with what Simmune predicts; details appear in the July 21 issue of PLoS Computational Biology.

Maryland, College Park, considers Aeromonas “provocative,” saying that it “teases us with its erratic pathogenic potential.” Its checkered history traces back more than a century when the first samples of Aeromonas were isolated, and sometimes misidentified as vibrios, he points out. Over many decades, it has been considered a cause of various conditions, including osteomyelitis, travelers’ diarrhea, and skin and other soft-tissue infections. At various junctures, notably in 1985, experts have argued that Aeromonas is not a human pathogen.

However, a Brazilian report earlier this year “may be the proof we’ve been waiting for,” Joseph says. It reviews an acute outbreak involving more than 2,000 individuals of gastrointestinal (GI) illnesses in 2004 in which several different species of Aeromonas were recovered in about 20% of the cases. Other more widely recognized pathogens, including Shigella and Vibrio cholerae, also were recovered in some of the cases. Aeromonas also continues to crop up as the likely pathogen for other medical conditions, including a case of necrotizing pneumonia for a swimmer in Portugal during the early 1990s and as a cause of skin wounds among a group of 22 Australian mud football players in 2002 and, more recently, a man who was mugged in Honolulu.

Perhaps the strongest case for Aeromonas being a human pathogen comes from examining its potential role in GI disease, according to Joseph. And, if anything, Plesiomonas is “a bit more severe” for the GI disturbances it causes, albeit with lower frequency, he says. “Aeromonas causes diarrhea, particularly among children and probably in adults, but we don’t have large outbreaks to prove it.” Possibly only some types are genuinely pathogenic, he speculates. “It’s reminiscent of Campylobacter in the 1960s, when people doubted it was really a human pathogen.”

The “taxonomic structure” of these two sets of microorganisms is “quite complex,” says symposium participant Geert Huys of the Universiteit Gent in Gent, Belgium. Moreover, some strains appear to have an array of virulence factors, according to Ashok Chopra of the University of Texas Medical Branch in Galveston. In terms of taxonomy, some strains are motile, while others are not, and there are 16 “phenotypic” but 18 “genotypic” species as well as other subspecies and other variants, Huys points out. “As far as we can tell, only a few of the strains are considered relevant human pathogens.”

One odd source of Aeromonas-related infections is through the medical use of leeches, according to Joerg Graf of the University of Connecticut in Storrs. The strains are part of the leech microbiota, sometimes as a pure culture but also mixed with Bacteroides species. To prevent such infections, patients who are treated with leeches also routinely receive antibiotics, he notes.

Whatever its status in clinics, Aeromonas gets credit for carrying “lots of virulence factors” along its genome, says Horneman of UM in Baltimore. The genome is 4.7 Mb, and in terms of coding sequences it is “closest” to Vibrio, Shewanella, and Plesiomonas species. Because it is found in diverse aquatic environments, it is “metabolically diverse,” she notes. “I am convinced that Aeromonas causes disease in humans, and it will be like Escherichia coli . . . with subsets of pathogenic strains.”

Jeffrey L. Fox